



Valve-gaping behavior of raft-cultivated mussels in the Ría de Arousa, Spain

Luc A. Comeau^{a,*}, Jose M.F. Babarro^b, Angeles Longa^c, Xose A. Padin^b

^a Fisheries and Oceans Canada, Gulf Fisheries Centre, Science Branch, P. O. Box 5030, Moncton, New Brunswick, E1C 9B6, Canada

^b Instituto de Investigaciones Marinas, CSIC, Eduardo Cabello 6, 36208 Vigo, Spain

^c Departamento de I+D, Consello Regulador Mexillón de Galicia, Spain

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ABSTRACT

We describe the valve-opening behavior of raft-cultivated mussels (*Mytilus galloprovincialis*) in the Ría de Arousa (Arousa estuary), Spain. Eight rope-grown mussels [mean \pm standard error (SEM), shell length 61.6 ± 2.1 mm] were connected to a non-invasive valvometry apparatus that monitored (one measurement min^{-1}) the magnitude of valve openness systematically over a 10 day period. It was found that valves were open $97.5 \pm 1.3\%$ percent of the time. Valve closures were not synchronized among the eight monitored mussels, suggesting that feeding cessation was physiologically-regulated rather than environmentally-mediated. The opening amplitudes that were most frequently observed were in the range of 60–90%, indicating that, when open, valves are usually opened relatively close to their maximum possible extent. The majority (7/8) of mussels displayed a circadian rhythm ($\tau = 24.0$ h) in valve opening amplitude. They tended to exhibit maximum valve opening during nighttime and minimum opening during daytime. It is possible that the light:dark cycle represents an environmental zeitgeber entraining an endogenous gaping rhythm in this bivalve.

1. Introduction

Over the past several decades, small sensors have been developed to monitor a range of parameters linked to a bivalve's health and its surrounding environment. These variables include heart beat rhythm (Braby and Somero, 2006; Burnett et al., 2013), body temperature (Andrewartha et al., 2015), and valve movements (Frank et al., 2007; Nagai et al., 2006). New engineering advances have also been developed to transform sensors into fully-automated and field-deployable monitoring devices. Recently, the natural behaviors of the ocean quahog (*Arctica islandica*) and fan mussel (*Pinna nobilis*) have been monitored using valve gape recorders in waterproof housings placed on the seabed (Ballesta-Artero et al., 2017; Garcia-March et al., 2016). Other devices adhere to the concept of biological early-warning systems. For example, the MolluSCAN eye is a highly specialized system that transfers valve movement data automatically through a mobile network (Andrade et al., 2016), allowing land-based servers to scan for abnormal behaviors linked to the presence of pollutants, such as trace metals or toxic microalgae (Tran et al., 2004; Tran et al., 2003). Similarly, sensors that monitor heart rhythm, valve opening, and other parameters were integrated recently into a field-deployable system that can transfer data continuously to cloud storage, thus allowing researchers to assess conditions remotely in real time. Andrewartha et al. (2015) described this system and the relevance of its use in sentinel

bivalves for assessing animal health and managing conditions in aquaculture farms.

The aim of this present study was to gain insight into the gaping behavior of the mussel *Mytilus galloprovincialis* being cultivated in the Galicia region (in NW Spain). It is noteworthy that the production of *M. galloprovincialis* in this region is approximately 267,000 t per year (Gosling, 2015). To provide substrate for growth, mussel ropes are suspended from large floating rafts anchored in numerous estuarine embayments (Rías) along the Galician coastline. In this paper, we describe the valve movements of *M. galloprovincialis* attached to ropes on this type of mussel raft.

2. Methods

2.1. Sensor connections

On 9 December 2015, *M. galloprovincialis* were collected from a commercial raft in the Ría de Arousa (Arousa estuary) (Fig. 1a). Mussels on a rope attached to the raft were detached carefully to avoid damaging the byssus gland or foot of the mussels. Mussels were then transported to the nearby Instituto de Investigaciones Marinas in Vigo, where they were held in four 191 tanks under the same conditions as described in Babarro and Fernández-Reiriz (2010). Tanks were continuously supplied with filtered (10 μm pore size) seawater (35 gl^{-1}

* Corresponding author.

E-mail address: luc.comeau@dfo-mpo.gc.ca (L.A. Comeau).

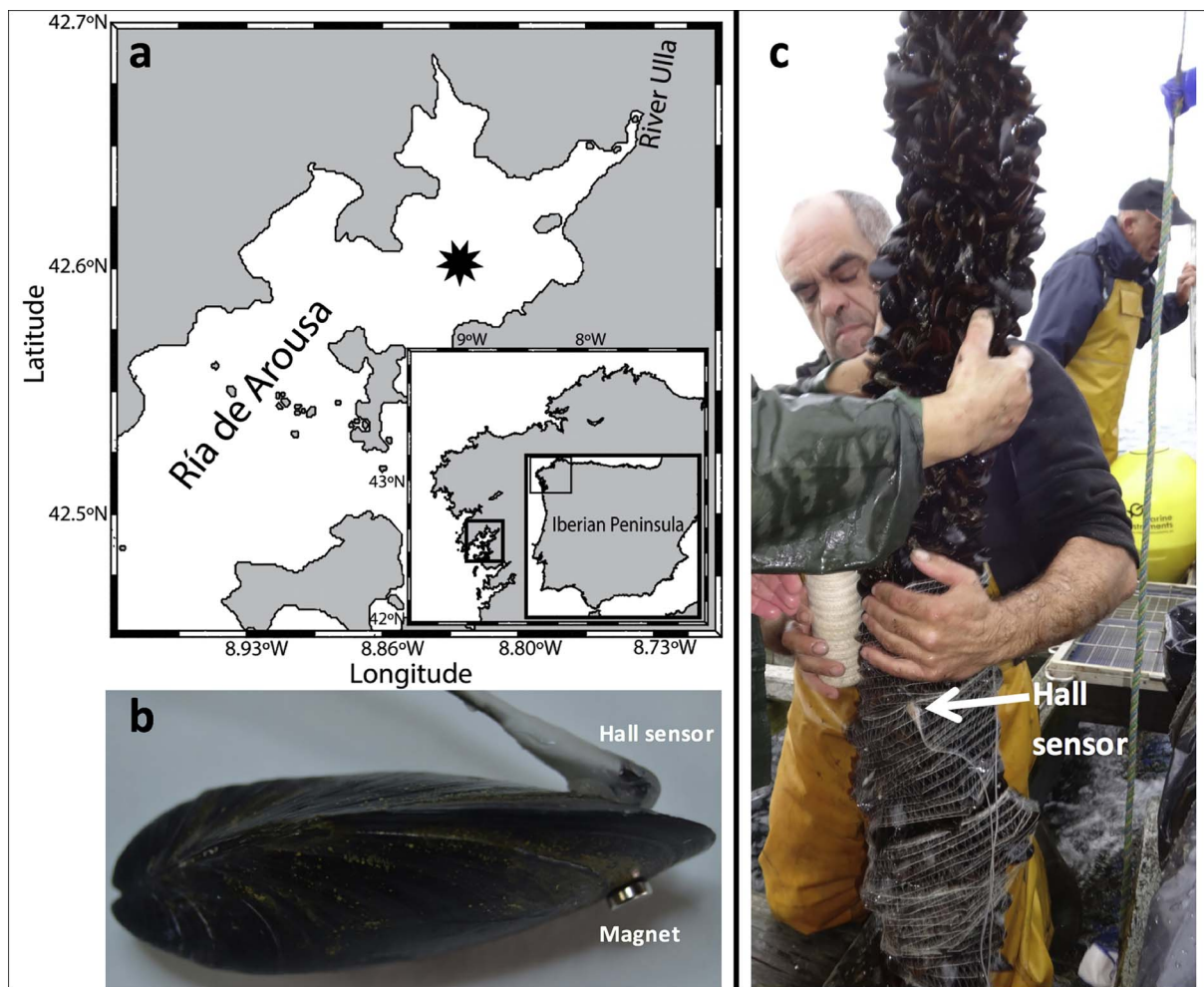


Fig. 1. (a) Map of study area showing the location of studied mussel (marked by ★) in the inner Ría de Arousa (Arousa estuary). (b) Photo of a single mussel (*Mytilus galloprovincialis*) equipped with a Hall sensor and magnet. (c) Photo of mussel rope holding the experimentally wired-mussels with cotton mesh being applied.

salinity, 15 °C) supplemented with a mixture of microalgae (Tahitian *Isochrysis* aff. *galbana*, T-ISO) and sediment collected from the seafloor below the mussel culture rafts (ratio of 40:60 microalgae:sediment, by weight). Particulate material concentration in the experimental tanks was maintained at 1.0 mg l^{-1} with an organic content of 50%, which simulated mean food availability in the water column along the Galician Rías coastline (Babarro et al., 2000).

On 11 December 2015, eight mussels (shell length $61.6 \pm 2.1 \text{ mm}$) were connected to a non-invasive valvometry system described in Nagai et al. (2006). A coated Hall element sensor (HW-300a, Asahi Kasei, Japan) equipped with a small electrical cable (1.5 mm diameter, 4.9 g per m) was attached to one valve using cyanoacrylate glue. Only the sensors (not the cables) were glued to the valve. A magnet (4.8 mm diameter by 0.8 mm high) was then glued to the other valve so that it was located on the opposite side of the Hall sensor (Fig. 1b). The magnet (0.1 g weight) and the Hall element (0.5 g weight) were both positioned at the posterior end of the animal. We assumed that these added weights would be inconsequential to the mussel because one live barnacle (6 mm diameter), a common epibiont on mussel shells (Doiron, 2008), weighs approximately 0.12 g. Mussels were returned to the holding tanks after the glue had set completely.

2.2. Mussel deployment

On 14 December 2015, the wired mussels were returned to a commercial raft in the Ría de Arousa, located in the inner portion of the estuary ($42^{\circ}36.02'N$, $8^{\circ}49.587'W$), and anchored at depths of 20–21 m.

At the time of the study, the raft had 500 suspended mussel ropes under production (each rope $\approx 12 \text{ m}$ in length). One of the peripheral ropes at the stern of the raft was selected for placement of the experimental mussels (Fig. 1c). Wired-mussels were equally-distributed between two depths (2 m and 7 m) on the rope. Each depth position held four mussels. A naturally-dissolvable cotton mesh was used to maintain the experimental mussels in place at the onset of the experiment, so that they could attach to the rope and to existing byssus webs of other mussels. Pieces of cotton mesh were also used to secure the wires of the eight Hall sensors (1 per mussel) running the length of the mussel rope.

The magnetic field (flux density) between each Hall sensor and associated magnet is a function of the gap distance between the two sides of the valve. This magnetic field was recorded in the form of output voltage using a dynamic-strain recording device (DC 204R, Tokyo Sokki Kenkyujo Co., Japan), which was powered by a battery-pack recharged daily by solar panels. The recorders were contained within weather-proof cases secured on a wooden beam of the raft, positioned approximately 1 m above the water surface. Gap monitoring began on 16 December 2015, following a 2-d acclimation period. Gap distance (amplitude) was measured once per minute for 10 d for each mussel. At the end of the monitoring period, the voltage data were converted to absolute opening amplitudes (magnitude of opening) by applying conversion algorithms specific to each sensor assembly (i.e., one for each mussel). To do this, the adductor muscle of each mussel was severed and a small calibration wedge (1–6 mm in height) was maneuvered between the two valves at the point farthest from the umbo (i.e., at the posterior edge of the animal where gaping is maximum). By

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